# Formulation Development and Comparative Evaluation of Ethosomal Gel of Azole Antifungal Agents

**A SYNOPSIS**
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**Synopsis of the Ph.D. thesis entitled**

# Formulation Development and Comparative Evaluation of Ethosomal Gel of Azole Antifungal Agents

## 1. INTRODUCTION:

According to a recent Global Burden of Disease report, cutaneous fungal infections are the fourth most common health problems which involve approximately one billion people worldwide. Fungal skin infections are substantially divided into three categories, namely dermatophytoses, pityriasis versicolor, and cutaneous candidiasis. Amongst these classifications, dermatophytes are the leading cause of superficial infections. It should also be noted that any skin surface can be prone to infection.

Skin infections are the most common type of fungal infections, and the prevalence of superficial fungal infections has grown over the past two decades. Recently, there has been an interest in the investigations on the development of new procedures for the better and more convenient treatment of patients with fungal infections. Drug delivery to the skin seems to be the best choice for superficial fungal diseases. The epidermis is the target place of pathogens for accumulation and proliferation. The treatment should include proper topical formulations that can release a sufficient amount of drug in therapeutic concentrations and permeate through higher layers of the skin, like the stratum corneum, to achieve suitable treatment. Recently, nanomedicine, by combining nanote-chnology, pharmaceutical science, and biomedicals with the application of nanoparticles, has shown a lot of abilities in the field of drug delivery. In the world of nano, with the interaction of nanocarriers in the sub-atomic states of skin layers, many efficacies are exhibited, such as overcoming the barriers. Liposomes, as the first approved nano-drug delivery system by FDA, have shown great efficacy due to their versatility, safety, biocompatibility, targeted delivery, high capacity, and capability to deliver both hydrophobic and hydrophilic molecules. For more than 60 years, these lipidic bilayers have been analyzed from multiple aspects and have shown stupendous advantages in many fields of medicine and pharmaceuticals as delivery systems. They have provided suitable circumstances for overcoming drug delivery deficiencies.

## ETHOSOMES

"Ethosomes are ethanolic liposomes" Ethosomes are systems containing soft vesicles composed of hydroalcoholic or hydroglycolic phospholipids, in which the concentration of alcohols or their combination (ethanol and isopropyl) is relatively high. High concentration of ethanol makes the ethosomal system unique. Ethosomes composed of ethanol in the range of 20% to 50 %. The size range of ethosomes may vary from nano metres to microns.

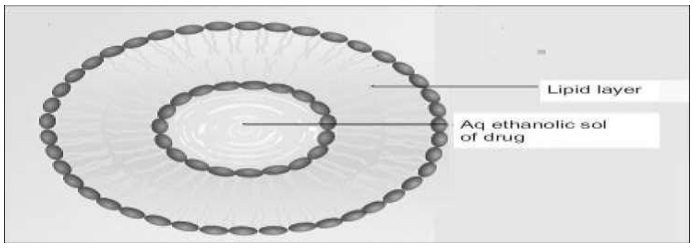
## TYPES OF ETHOSOMAL SYSTEMS

There are 3 types of ethosomal systems –

**1. Classical Ethosomes** Classical ethosomes are composed of phospholipids, water and high concentration of ethanol (40%). Drugs having molecular weight ranging from 130,077 Da to 24kDa can be entrapped in classical ethosomes.

**2. Binary Ethosomes** Binary ethosomes can be prepared by adding another type of alcohol to the classical ethosomes. Propylene glycol (PG) and isopropyl alcohol (IPA) are most commonly used alcohols.

**3. Transethosomes** It contains basic components from classical ethosomes and a penetration enhancer(surfactant). Developed to combine the advantages of classical ethosomes and transferosomes to produce transethosomes. Transethosomes are advanced type of ethosomes as they have the advantages of both i.e classical ethosome and transferosomes.




**Structure of Ethosome**

**DIFFERENT TYPES OF ADDITIVES USED IN FORMULATION OF ETHOSOME**

| Class | Example | Uses |
|---|---|---|
| Phospholipid | Soya phosphatidyl choline<br>Egg phosphatidyl choline<br>Dipalmityl phosphatidyl choline<br>Distearyl phosphatidyl choline | Vesicles forming component |
| Polyglycol | Propylene glycol<br>Transcutol RTM | As a skin penetration enhancer |
| Alcohol | Ethanol<br>Isopropyl alcohol | For providing the softness for vesicle membrane<br>As a penetration enhancer |
| Cholesterol | Cholesterol | For providing the stability to vesicle membrane |
| Dye | Rhodamine-123<br>Rhodamine red<br>Fluorescene Isothiocynate (FITC)<br>6- Carboxy fluorescence | For characterization study |
| Vehicle | Carbopol Đ934 | As a gel former |

Ethosomes were reported to improve the skin delivery of various drugs]. Ethanol is known as an efficient permeation enhancer that is believed to act by affecting the intercellular region of the stratum corneum. Its inclusion in liposomes to form ethosomes has already been investigated. Transdermal drug delivery systems offer many advantages over their corresponding classical oral, injectable, and inhaler systems, including improving the systemic bioavailability of drugs because the first-pass metabolism by the liver and digestive system is avoided, and achieving a controlled constant drug delivery profile, which is especially important for those suffering from nocturnal attacks and need a longer duration of therapeutic action from a single application. Despite the many advantages of the skin as a site of drug delivery, only few drugs are currently in the market for transdermal delivery system. The primary

reason for this is the low permeability of drugs in the stratum corneum, as stratum corneum (outermost layer) acts as the main barrier in the skin. In general the highly organized crystalline lipid lamellae play an essential role in the barrier properties of the stratum corneum. Many techniques have been aimed to disrupt and weaken the highly organized intercellular lipids in an attempt to enhance drug transport across the intact skin or to increase the driving force for permeation of drugs across this skin barrier. The vesicles have been well known for their important in cellular communication and particle transportation for many years. Researchers have understood the properties of vesicle structures for use in better drug delivery within their cavities that would allow for tagging the vesicle for cell specificity. The ethosomes more advantages when compared to transdermal and dermal delivery. It delivers large molecules such as peptides, protein molecules. Simple method for drug delivery in comparison to Iontophoresis and Phonophoresis and other complicated methods. Low risk profile- The technology has no large-scale drug development risk since the toxicological profiles of the ethosomal components are well documented in the scientific literature. High patient compliance as it is administrated in semisolid form (gel or cream) and various application in Pharmaceutical, Veterinary, Cosmetic field.

**METHOD OF PREPARATION**

There are two methods which can be used for formulation and preparation of ethosomes. Both of these are very simple and convenient and do not involve any sofisticated instrument or complicated process. Methods are

1. Hot method.
2. Cold method

**HOT METHOD**

In this method phospholipid is dispersed in water by heating in a water bath at 400 °C until a colloidal solution is obtained. In a separate vessel ethanol and propylene glycol are mixed and heated to 400 °C. Once both mixtures reach 400 °C, the organic phase is added to the aqueous one. The drug is dissolved in water or ethanol depending on its hydrophilic/ hydrophobic properties. The vesicle size of ethosomal formulation can be decreased to the desire extent using probe sonication or extrusion method.

**COLD METHOD**

In this method phospholipid, drug and other lipid materials are dissolved in ethanol in a covered vessel at room temperature by vigorous stirring with the use of mixer. Propylene glycol or other polyol is added during stirring. This mixture is heated to 300 °C in a water bath. The water heated to 300 °C in a separate vessel is added to the mixture, which is then stirred for 5 min in a covered vessel. The vesicle size of ethosomal formulation can be de-creased to desire extendusing sonication or extrusion method. Finally, the formulation is stored under refrigeration.

**METHODS FOR THE CHARACTERIZATION OF ETHOSOMAL FORMULATION**

| Parameters | Methods |
|---|---|
| Vesicle shape (morphology) | Transmission electron microscopy<br>Scanning electron microscopy |
| Entrapment efficiency | Mini column centrifugation method<br>Fluorescence spectrophotometry |
| Vesicle size and size distribution | Dynamic light scattering method |
| Vesicle Skin interaction study | Confocal laser scanning microscopy<br>Fluorescence microscopy<br>Transmission electron microscopy<br>Eosin-Hematoxylin staining |
| Phospholipid-ethanol interaction | $^{31}$P NMR<br>Differential scanning calorimeter |
| Degree of deformability | Extrusion method |
| Zeta potential | Zeta meter |
| Turbidity | Nephalometer |
| *In vitro* drug release study | Franz diffusion cell with artificial or biological membrane, Dialysis bag diffusion |
| Drug deposition study | Franz diffusion cell |
| Stability study | Dynamic light scattering method<br>Transmission electron microscopy |

## ADVANTAGES OF ETHOSOMAL DRUG DELIVERY

In comparison to other transdermal & dermal delivery systems-

- Enhanced permeation of drug through skin for transdermal drug delivery.
- Delivery of large molecules (peptides, protein molecules) if possible.
- It contains non-toxic raw material in formulation.
- High patient compliance-The ethosomal drug is administrated in semisolid form (gel or cream) hence producing high patient compliance.
- The Ethosomal system is passive, non-invasive and is available for immediate commercialization.
- Ethosomal drug delivery system can be applied widely in Pharmaceutical, Veterinary, Cosmetic fields. Simple method for drug delivery in comparison to iontophoresis and phosphophoresis and other complicated methods.

## APPLICATION OF ETHOSOMES AS A DRUG CARRIER

| Drug | *Results* |
|---|---|
| NSAIDS (Diclofenac) | Sel↓ective delivery of drug to desired side for prolong period of time |
| Acyclovir | Increase skin permeation↓<br>Improved in biological activity two to three times↓<br>Improved in Pharmacodynamic profile↓ |
| Insulin | Significant decrease in blood glucose level↓<br>Provide control release ↓ |
| Trihexyphenidyl hydrochloride | Improved transdermal flux↓<br>Provide controlled release↓<br>Improved patient compliance↓<br>Biologically active at dose several times lower than the currently used formulation↓ |
| DNA | Better expression of genes↓<br>Selective targeting to dermal cells ↓ |
| Antibiotic<br>Cannabidol<br>Erythromycin | Improved skin deposition↓<br>Improved biological activity↓<br>Prolonging drug action ↓ |
| Bacitracin | Improved dermal deposition↓<br>Improved intracellular delivery↓<br>Increased bioavailability ↓ |
| Anti-HIV agents<br>Zidovudine<br>Lamivudine | Improved transdermal flux↓<br>Improved in biological activity two to three times↓<br>Prolonging drug action↓<br>Reduced drug toxicity↓<br>Affected the normal histology of skin ↓ |
| Azelaic acid | Prolong drug release ↓ |
| Ammonium glycyrrhizinate | Improved dermal deposition exhibiting sustained release↓<br>Improved biological anti-inflammatory activity ↓ |

## 2. HYPOTHESIS:

Cutaneous fungal infections are the fourth most common health problem, which involves approximately one billion people worldwide. Drug delivery to the skin seems to be the best choice for superficial fungal infections. Topical formulations can release a sufficient amount of drug in therapeutical concentrations and permeate higher layers of the skin like the stratum corneum. As the outermost layer of the epidermis, the stratum corneum prevents the drug from penetrating the skin. Ethosomes, as topical drug delivery systems to the skin, can show various functions depending on their size, lipids and cholesterol components, the percent of ingredients, lamellarity, and surface charge. Ethosomes can increase permeation through the stratum corneum, decrease systemic effects with their localizing actions, and overcome many dermal drug delivery obstacles. Antifungal drugs, such as clotrimanazole, econazole, fluconazole, ketoconazole, terbinafine hydrochloride, tolnaftate, and miconazole entrapped in ethosomes have indicated improved skin penetration and localizing effects.

**SELECTION OF ETHOSOMES AS DELIVERY SYSTEM**

- In ethosomes, ethanol present in high concentration i.e. 20% to 50% which act as penetration enhancer and increases the skin permeability.
- Ethosomes are selected because they are suitable for all types of molecule i.e. hydrophilic, lipophilic and amphiphilic.
- Ethosomes are capable to enable the drug to reach the deep skin layers or systemic circulation.
- Ethosomal drug delivery is a simple method as compared to iontophoresis and sonophoresis.
- As compared to liposomes, ethosomes exhibit high encapsulation efficiency, flexibility, higher in-vitro and in-vivo skin permeability and skin deposition ability for wide range of drugs.
- The incorporation of drug in the ethosomes will provide sustaining action of the drug leading to an increase in the bioavailability of the drug. This will also lead to an increase in patient compliance due to decrease in frequency of dosing.

# 3. REVIEW OF LITERATURE

**Maha A *et. al.*, 2022** investigated the development of sustained release transdermal carvedilol-loaded ethosomal formulation. CAR- ethosomal formulation was prepared by cold method with1% lipid, 50mg Carvedilol, 30% ethanol (v/v) and sonication time 3 min. The prepared formulation was characterized for vesicle size, polydispersity index (PDI) and entrapment efficiency (EE%). The formula showed small vesicle size (46.75±9.41nm) and high EE% (97%). Accordingly, it was subjected to stability study, TEM and zeta potential analysis. The formula showed good physical stability at refrigeration temperature after 90 days storage period as well as good zeta potential of +38.75mV. Their formula exhibited spherical unilamellar structure under TEM. Consequently, our formula was made as gel using HPMC polymer and was tested for pH, spreadability, drug content, skin permeation and pharmacokinetic study by HPLC-MS technique. Plasma drug levels were measured for rats after administration of both ethosomal gel and oral carvedilol tablet. The pharmacokinetic parameters were calculated and compared between the transdermal and oral dosage forms. Our formula showed lower t max and higher $AUC_{0-24}$ than oral carvedilol tablet. Our findings affirm the potential of ethosomes as new vesicular carriers in sustained transdermal management of common cardiovascular conditions.

**Nasr AM *et. al.*, 2022** formulated and evaluated valsartan (VLT) ethosomes to prepare an optimized formula of VLT-entrapped ethosomes that could be incorporated into a sustained release transdermal gel dosage form. The formulation of the prepared ethosomal gel was investigated and subjected to in vitro drug release studies, ex vivo test, and in vivo studies to assess the effectiveness of ethosomal formulation in enhancing the bioavailability of VLT as a poorly soluble drug and in controlling its release from the transdermal gel dosage form.

**Mehwish Andleeb *et. al.*, 2021** extrated *Achillea millefolium* L. (AM) extract and s tested at first for phytochemical analysis, antioxidant activity, total phenolic and flavonoid content, and FTIR examination. The nanoethosomes containing AM extract were synthesized and characterized by size, surface charge, and morphology, and entrapment efficiency (EE) was determined. The optimized nanoethosomes were then incorporated to develop a topical gel formulation and subjected to skin for permeation, pH, viscosity, and organoleptic evaluation for up to three months.

The AM ethanolic extract demonstrated 88% free radical scavenging activity and notable phenolic and flavonoid contents of up to 123 mg GAE/g and 42 mg QE/g, respectively. The optimized nanoethosomes encapsulated with AM extract (240 nm) were spherical in shape, with −31.1 mV of surface charge, and showed considerable entrapment efficiency (90%). Furthermore, the selected topical gel remained stable during the study period. The Exvivo permeation study of ethosomal gel showed the highest release percentage of 79.8%.

**Rekha B *et. al.*, 2018** focused to enhance transdermal as well as subdermal delivery of Atorvastatin (ATV) using ethosomal system which composed of phospholipid, ethanol and water. Effect of different formulations on characterization and in-vitro drug release was conducted and ethosomes containing 30% w/w ethanol showed better entrapment efficiency ,small vesicle size, high transdermal flux and maximum skin penetration .Further sonication reduced the size of the vesicles and vesicles were more uniform in size and shape and showed better characterization. Release kinetic of ATV from ethosomes was found to be zero order while other formulations showed far away from ethosomes. Ethosomes were found stable in refrigeration and room temperature during storage of 8weeks. It is concluded from the present investigation that ethosomes increase the transdermal flux and gives predictable release rate of atorvastatin and hence ethosomal system is a promising candidate for delivery of atorvastatin into and through the skin.

**Priyanka Parmar *et. al.*, 2016** developed ethosomes of clotrimazole by using different concentration of soya lecithin and then optimized formulation batch was selected by determining Size and surface morphology , drug entrapment efficiency (DEE),in-vitro release study and the best formulation was used to prepare gel by using carbopol 934 as a gelling agent , pH and Viscosity were determined of Ethosome entrapped gel. The formulated ethosomal gel obtained was exploited to achieve a zero order release profile of Clotrimazole. DEE was obtained between 52.6 to 60% in best formulations.

**A.Sunil Kumar Reddy *et. al.,* 2016** prepared and evaluated Simvastatin ethosomes containing different concentrations of ethanol and phospholipids by sonication for size reduction of vesicles. Designed ethosomes are characterized for size, shape, entrapment efficiency, release study and stability study. The effect of sonication also studied on the characterstics of simvastatin ethosomes.

**Zhenwei Yu *et. al.,* 2016** developed ethosomes loaded with Cryptotanshinone(CPT) and formulate them as a topical gel for the treatment of acne. Ethosomes were prepared and evaluated for vesicle size, CPT loading and encapsulation efficiency. Optimized ethosomes were formulated as Carbomer 974 gels and compared with conventional hydroethanolic gels for transdermal permeation and skin deposition in vitro. The anti-acne activity and skin irritation of the gel was investigated in rabbits. The transdermal flux and skin deposition and skin deposition of the optimized ethosomal gel were 2.5 and 2.1 times those of conventional gels. This study demonstrates that ethosomal formulations an effective dermal delivery system for CPT.

**Dileep P. *et. al.,* 2015** developed different formulations of ethosomes of Itraconazole using different concentrations of polymer i.e. soya lecithin (2.5%), and ethanol (20-50%). The best achieved ethosomal vesicles suspension, was incorporated into carbopol gel (1%, 1.5%, 2%w/w). Gel was evaluated with different parameters like organoleptic character, pH, entrapment efficiency, drug content. Ethosomes containing 30%w/w ethanol and prepared by sonication showed highest value with respect to all other formulations.

## 4. AIM AND OBJECTIVES:

The current research work aims to fulfill following objectives described in bulleted points below, a brief detail of the methods to be adopted is given in the section describing plan of work.

- To perform preformulation studies on antifungal drugs.
- To perform Spectral characterization and interactions studies among various excipients.
- To develop ethosomal gel by cold and hot methods.
- To evaluate prepared gel on the basis of physical and formulation charectrastics.
- To perform *In vitro* evaluation of prepared gel.
- To perform *In-vitro* Permeation Study and Stability Studies as per ICH Guidelines

## 5. METHODOLOGY

The proposed research work shall be carried using well establish and documented method. Various studies like synthesis and validation shall be undertaken. Moreover, feasibility of scale up of various methods will also be considered. Various pharmacological procedures shall be validated and established. Moreover *in-vitro* assessment and *in-vivo* performance studies will be under taken.

**Plan of Work**

- Exhaustive literature survey.
- Procurement of Drugs and Chemicals.
- **Preformulation Studies**
    - Solubility
    - Melting Point
    - Partition Coefficient
    - U.V. Spectroscopy
    - I.R. Spectroscopy
    - Drug Excipient Study
- **Preparation of Ethosomes**
    - Cold Method
    - Hot Method
- **Evaluation of Ethosomes**
    - Particle Size and Particle Distribution
    - Zeta Potential
    - Scanning Electron microscopy
    - Determination of Vesicle Shape
    - Determination of Drug Entrapment Efficiency
    - Determination of Drug Content
- **Preparation of Ethosomal Gel**
- **Evaluation of Ethosomal Gel**
    - Physical Appearance and Homogeneity
    - Rheological Studies
        - Viscosity

        - Spreadability
    - Extrudability Studies
    - Skin irritation Study
    - Antifungal Evaluation
- ***In-vitro* Permeation Study**
- **Stability Studies as per ICH Guidelines**
- Compilation of data, statistical treatment of data and presentation of dissertation.
- Thesis writing and publication.

## Address of related website:

- http://www.sciencedirect.com/
- http://www.ncbi.nlm.nih.gov/pubmed/
- http://onlinelibrary.wiley.com/browse/publications?type=journal
- http://www.doaj.org/
- http://search.proquest.com/

## Major inputs: (infrastructure for research work):

We have the necessary facilities for preparation of the ethosomal gel systems and their preliminary characterization these include high speed stirrers, low temperature centrifuges, Rota evaporator and Lyophilizer. For chemical characterization the institute has facilities of FTIR, UV spectrophotometer. For stability studies stability chamber have been initiated by the host institute. The institute is also equipped with all basic necessities and other devices like rotary vacuum evaporator to facilitate the smooth start of the project. Other specific characterization can be easily done on rent basis at **Indian Institute of Technology, Kanpur** and **Central Drug Research Institute, Lucknow**.

## Proposed Chapter Scheme:

1. Introduction
2. Literature Review, Research envisaged and Plan of Work
3. Material and Methods
4. Formulation development
5. In vitro Assessment
6. Result and Discussion
7. Summary and Conclusion

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